



IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF :  
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FOR: EFFICIENT PRODUCTION :  
METHOD OF ASCOPYRONE P

DECLARATION UNDER 37 C.F.R. 1,132

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I, Kazuhiro Yoshinaga, am a doctor of agriculture and one of the inventors of the present invention.

I have a good grasp of the method of manufacturing Ascopyrone P developed by Ahmad (The Swedish University of Agricultural Sciences, Sweden, pages 1-34, 1995) and Elsser et al (WO02/26060A1). I have conducted the following experiments to demonstrate that the production yield of Ascopyrone P in the method is very low and that Ascopyrone P cannot be produced by the method at an industrially significantly high yield as seen in the present invention.

Experimental method

Zero point three five milliliters of each buffer (pH 2.2 to 13.3) in Table-B was added to 0.35 ml of 1,5-D-anhydrofructose aqueous solution (50 mg/ml), and the resulting mixture was fed to a test tube having a screw cap to be heated at a predetermined temperature for a predetermined time. For heating, a water bath was used to heat the mixture up to 100°C and a silicon oil bath was used to heat it up to 110 to 130°C. After heating, 0.35 ml of water was added to the reaction solution. Five microliters of the solution was taken out, 495 µl of water was added. The mixture was analyzed by high-speed liquid chromatography.

Table B buffer fluids

Buffer	Concentration	pH
Lactic acid	1M	2.2
Lactic acid/Sodium lactate	1M	4.0
Citric acid/Sodium hydroxide	1M	6.9
Disodium hydrogen phosphate Potassium dihydrogen phosphate	500MM	7.5
Potassium phosphate	500MM	8.8
Sodium hydroxide	1M	13.3

Table C-1 (reaction temperature: 25°C)

		Time (min.)					
		5.0	30.0	60.0	120.0	240.0	1620.0
pH	2.2	0.0	-	0.0	0.0	0.0	0.0
	4.0	0.0	-	0.0	0.0	0.0	0.0
	6.9	0.0	-	0.0	0.0	0.0	0.0
	7.5	0.0	-	0.0	0.0	0.0	0.0
	8.8	0.0	-	0.0	0.0	0.0	0.1
	13.3	2.3	4.1	2.9	2.1	1.6	0.0

Table C-2 (reaction temperature: 50°C)

		Time (min.)				
		1	2.5	5.0	60.0	120.0
pH	2.2	-	-	-	-	-
	4.0	-	-	-	-	-
	6.9	-	-	-	-	-
	7.5	-	-	-	-	-
	8.8	-	-	-	-	-
	13.3	6	7.5	7.4	1.4	0.3

Table C-3 (reaction temperature: 75°C)

		Time (min.)			
		5.0	60.0	120.0	180.0
pH	2.2	0.0	0.0	0.0	0.0
	4.0	0.0	0.0	0.0	4.9
	6.9	0.0	1.1	2.7	4.2
	7.5	0.0	3.3	4.8	3.9
	8.8	0.5	3.3	1.7	-
	13.3	4.2	0.0	0.0	-

Table C-4 (reaction temperature: 90°C)

		Time (min.)			
		5.0	60.0	120.0	180.0
pH	2.2	0.0	0.5	0.8	0.8
	4.0	0.3	15.6	23.1	23.5
	6.9	0.7	8.4	7.4	5.5
	7.5	3.3	4.9	2.0	0.7
	8.8	3.8	1.4	0.5	0.2
	13.3	-	-	-	-

Table C-5 (reaction temperature: 100°C)

		Time (min.)						
		5.0	60.0	120.0	180.0	240.0	300.0	360.0
pH	2.2	0.0	1.0	3.8	5.9	7.1	7.9	5.9
	4.0	1.1	30.0	27.0	-	-	-	-
	6.9	1.6	6.6	8.4	-	-	-	-
	7.5	6.1	1.9	1.3	-	-	-	-
	8.8	4.3	0.4	0.3	-	-	-	-
	13.3	0.0	0.0	0.0	-	-	-	-

Table C-6 (reaction temperature: 110°C)

		Time (min.)		
		5.0	60.0	120.0
pH	2.2	0.1	-	-
	4.0	4.2	-	-
	6.9	5.1	-	-
	7.5	6.8	-	-
	8.8	4.1	-	-
	13.3	-	-	-

Table C-7 (reaction temperature: 120°C)

		Time (min.)		
		5.0	60.0	120.0
pH	2.2	0.5	-	-
	4.0	17.0	-	-
	6.9	9.1	-	-
	7.5	6.6	-	-
	8.8	1.6	-	-
	13.3	-	-	-

Table C-8 (reaction temperature: 130°C)

		Time (min.)		
		5.0	60.0	120.0
pH	2.2	1.3	-	-
	4.0	35.7	-	-
	6.9	11.7	-	-
	7.5	-	-	-
	8.8	-	-	-
	13.3	-	-	-

### Results and Discussions

The conversion ratio of 1,5-D-anhydrofructose to Ascopyrone P, i.e., % yield of Ascopyrone P obtained in each of the experiments is shown in Tables C-1 to C-8.

As shown in the lower rows of Table C-1, the maximum production yield of Ascopyrone P was 4.1 % (25°C, pH13.3, 30 minutes) even under the pH condition of Ahmad (neutral to

alkaline) at 25°C even when the reaction time was extended up to 27 hours (1620 min).

As shown in the lower rows of Tables C-2 to C-8, the maximum production yield of Ascopyrone P was 7.5 % (50°C, pH13.3, 2.5 minutes) under the pH condition of Ahmad when the reaction temperature was changed to the elevated temperature such as 70°C disclosed in Elsser et al.

As shown in the upper rows of Tables C-1 to C-4, the maximum production yield of Ascopyrone P rose to 23.5 % (90°C, pH = 4.0, 180 min) when the pH condition of Ahmad was changed to an acidic range disclosed in Elsser et al though the reaction time was 180 minutes.

As shown in the upper rows of Tables C-5 to C-8, the maximum production yield of Ascopyrone P was 35.7 % (130°C, pH4.0, 5 minutes) under the reaction conditions of the present invention not disclosed by Ahmad and Elsser et al, that is, an acidic range and a temperature of 100°C or higher when the reaction time was extremely short (5 minutes). Even when the reaction temperature was 100°C and the reaction time was 120 minutes, a yield of 27.0 % (pH4.0) was obtained which is higher than the maximum production yield (23.5 %) shown in the upper rows of Tables C-1 to C-4.

The undersigned declares further that all statements made herein of his own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Further declarant saith not.

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9/17/2008

Date